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Chapter

Probiotics and Bioremediation

Qomarudin Helmy, Edwan Kardena and Sri Gustiani

Abstract

Increased environmental contamination leads to a progressive decline in environmental quality. Probiotics play a role as remediation agents which are expected to be able to help the host in responding to environmental changes. Probiotics are live microbial feed supplements that favorably affect host (humans, animals, plants) by increasing the balance of intestinal microbes. Probiotics are used in livestock/aquaculture to improve growth performance through improved utilization of nutrients, reducing disease, also developing the immune system. In addition to feed supplements, certain types of probiotics act as bioremediation or decomposing agents of hazardous substances. The bioremediation system is the use of microorganisms (bacteria, fungi, yeast, and algae) or microbial products to degrade, reduce, or remove pollutants in the environment although in some cases plants are also utilized for this purpose called phytoremediation. When bioremediation occurs, enzymes produced by microorganisms modify toxic pollutants by changing the chemical structure of pollutants. This event is called biotransformation. In many cases, biotransformation leads to biodegradation, when toxic pollutants are degraded, the structure becomes simpler, and ultimately becomes harmless and non-toxic metabolites, that called mineralization.

Keywords: probiotics, bioremediation, aquaculture, livestock, agriculture

1. Probiotics: small creatures that do big things

Bacteria are often associated with diseases or with something that is frightening and disgusting. But along with advances in technology, bacteria today are not considered only as an enemy but can also be friends. Basically bacteria are microorganisms that is part of nature that are found almost everywhere on the earth's surface, atmosphere, also the upper atmosphere [1–3]. The shape is very small, cannot be seen by the eye but its existence is very important to maintain environmental balance. Microorganisms are usually considered to include all prokaryotes, protists and microalgae. Fungi, especially those that are small and do not form hyphae, can also be considered as a part of it, although many do not agree. Most people assume that what can be considered microorganisms are all very small organisms that can be bred in petri dishes or incubators in the laboratory and capable of reproducing themselves by mitosis [4].

Collection of beneficial microbes known as probiotics, a word derived from the Greek word that means for life. Among the researchers there were slight differences of opinion regarding what could be called probiotics, including:

- Probiotics are compounds produced by a microorganism that can stimulate the growth of other microorganisms, so it is the opposite of antibiotics [5].

- Probiotics are extracts from tissues that can stimulate the growth of microorganisms [6].

- Probiotics are organisms and substrates that have an influence on the balance of microbiota in the digestive system [7].

- Probiotics are living microorganisms that when consumed by the host will have a beneficial effect on them by improving the microbiota environment that exists in the digestive system [8].

- The current definition of probiotics was formulated by FAO/WHO in 2001 as living microorganisms which, if given in sufficient quantities, provide health benefits to the host [9].

Probiotics can be found in several products depending on the intended use. They can be marketed as food, medical food, food supplements or medicine. In general, probiotics are marketed as food supplements e.g. pill/tablet products or as food items, e.g., Yogurt, Kefir. Some probiotic organisms including *Lactobacillus* sp., *Streptococcus* sp., and *Bifidobacterium* sp. have been "generally recognized as safe" status, which means that they are additives that are permitted in food substances. The term probiotic was originally intended as a supplement used to improve or prolong human life or health by providing a sufficient amount of good microorganisms into the body through food. Having recognized the positive benefits of probiotics on human health, many practitioners and researchers have begun to try and apply probiotics to livestock, agriculture, and fisheries.

2. Probiotics in Aquaculture, Animal Husbandry, and Agriculture

Probiotics are actually used not only for human, but also fisheries, agriculture, and animal husbandry with the aim of increasing yield productivity and decomposing waste. In livestock farming, increased production can be achieved by optimizing environmental conditions, getting the right stocking density, improving seed quality and providing good quality feed. In addition, it can also be done through efforts to reduce mortality rates and increase individual growth rates. In intensive livestock farming, feed is the highest component of production costs, about 60–70% of operational costs [10]. The digestive system plays a vital role in the extraction of nutrients from feed and their absorption to be used by body cells. The main key that occurs in the digestive system is its ability to digest food which allows nutrients to be absorbed by the body. The digestibility value of a food ingredient illustrates the ability of livestock to digest a food and the digestibility itself is determined by the quality of the food provided. The digestive system plays a vital role in the extraction of nutrients from feed and their absorption to be used by body cells. The main key that occurs in the digestive system is its ability to digest food which allows nutrients to be absorbed by the body. Digestion is a chemical process and fermentation by microbiota in the intestine. The interaction between microbiota and nutrition is very complex. Microbiota and its metabolic products can affect the digestion and absorption of nutrients by the host. In other words, microbial balance in the digestive system plays an important role for health, feed digestibility, and production efficiency.

There are three suggested probiotic working mechanisms, namely:

1. Suppress harmful microbial populations through competition by producing antimicrobial compounds or through nutrition competition and attachment sites on the intestine wall,

2. Stimulating immunity through increased levels of antibodies or macrophage activity, and

3. Changing microbial metabolism by increasing or decreasing the activity of certain enzymes.

The growing livestock farming industry has a relationship with the presence of bacteria in the environment. An intensive cultivation system without good management will have a negative effect. For example, in intensive aquaculture farming with excessive feeding, maintenance of fish with high stocking densities without good management will cause disease for the fish. The resulting waste in the form of high organic matter will cause bacteria to flourish and ultimately reduce fish production and also damage the environment. Other problems with increasing intensive aquaculture activities are eutrophication, increased sedimentation and excessive plankton growth. This can result in high levels of ammonia in water and lack of oxygen.

Biological processes that occur in nature including the cultivation environment must be maintained in balance so that the quality of the environment is maintained well. One appropriate way is to use probiotics. Probiotics itself is a collection of beneficial microorganisms that help the process of absorption of food, can increase endurance and help improve environmental quality. Microorganisms that exist in probiotics are usually bacteria such as *Lactobacillus* sp., *Bacillus* sp., *Micrococcus* sp., Nitrobacter and Nitrosomonas. Probiotics can be given in various ways, through aquaculture feed or directly stocked into the aquatic environment. In probiotics itself there are several types of bacteria that can help the digestive process better such as *Lactobacillus* sp. These bacteria are able to maximize the absorption of food in the intestines of fish so that not much food is wasted and fish growth becomes faster. Under these conditions the amount of feed given is more efficient but provides maximum results. This can occur because the bacteria with its mechanism produces digestive enzymes such as amylase, protease, lipase to break down carbohydrates, proteins, fats in the feed so that it is more quickly absorbed by the body. Other probiotics with the type Nitrobacter and Nitrosomonas are able to decipher the rest of the remaining feed or feces that settles at the bottom so that the quality of water in ponds or ponds for the better. Stools and food waste that accumulate and not decompose will result in high levels of ammonia and sulfides which can be toxic to aquatic organisms.

The presence of beneficial microorganisms in aquaculture activities has been proven through several studies [11–15]. Fish feed with additional probiotics turned out to be able to increase the growth of catfish better than ordinary feed. Application of microorganisms to the environment is also shown by giving probiotics in the water from shrimp ponds that show a decrease in ammonia levels and maintain pH in neutral conditions compared to without giving probiotics. The antimicrobial role released by probiotic bacteria also has a role to prevent the emergence of disease, thereby increasing the resistance of fish or livestock to disease [16–20]. The presence of beneficial microbes that are naturally present in the intestine provides an opportunity and possibility to isolate and reproduce them, which are then reintroduced into the digestive system and used as probiotics (**Table 1**).

The application of probiotics in agriculture has been widely used, especially as a starter in making organic fertilizer. With this probiotic can accelerate the manufacture of organic fertilizer, compost for example naturally will take 3-6 months, but with probiotic technology only takes 3-4 weeks. Inoculation of probiotic microorganisms can be used as an alternative in overcoming the scarcity of inorganic fertilizers due to the high price and availability of fertilizers in certain areas. Inoculation

Subject	Probiotics organism	Results	Application	Reference
Aquaculture				
Shrimp (*Litopenaeus vannamei*)	Bacteria: *Bacillus thuringiensis*, *Bacillus megaterium*, *Bacillus polymyxa*, *Bacillus licheniformis* and *Bacillus subtilis* Yeast: *Debaryomyces hansenii*, *Rhodotorula* sp. Algae: *Chaetoceros* sp.	Post-larval stage of *L. vannamei* treated with either bacteria and yeasts or bacteria, yeasts and Chaetoceros exhibited increases ($P < 0.05$) in growth and survival as compared to controls.	Food pellets in the form of microencapsulated beads using sodium alginate	[11]
Striped catfish (*Pangasianodon hypophthalmus*)	*Bacillus amyloliquefaciens* 54A and *B. pumilus* 47B	The average weight gain of fish fed probiotics at 5×10^8 CFU/g significant higher than control after 90 days of feeding, but there was not significant effect on feed conversion ratio and specific growth rate.	Mixed with food pellets	[12]
Salmon (*Salmo salar* L.) and Trout (*Oncorhynchus mykiss*)	*Carnobacterium inhibens* K1	Increase appetite and feeding efficiency and increase resistance to *A. salmonicida*, *V. ordalli* and *Y. ruckeri*	Mixed with food pellets	[13]
Fresh water prawn (*Macrobrachium rosenbergii*)	Commercial probiotic: Zymetin (*Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus*, Beer yeast). Super PS (*Rhodobacter* sp. and *Rhodococcus* sp.)	The production of probiotics treated pond was always higher than without probiotics treated ponds, highest growth and production were found when Zymetin and Super PS were used together.	Mixed with food pellets	[14]
Sea bream larvae (*Sparus aurata*)	*Lactobacillus fructivorans* and *Lactobacillus plantarum*	The combination of probiotics (80:20 w/w) through dry or live feed promoted the sea bream larvae (*Sparus aurata*) intestinal microflora changes that contribute to reducing mortality in both sea bream larvae and fries.	Live feed and mixed with food pellets	[15]

Subject	Probiotics organism	Results	Application	Reference
Animal husbandry				
Pig	*Enterococcus faecium* SF 68	Positive effect of probiotics consumption on the digestive tract thus decreasing the diarrheic diseases that are frequent in the pig husbandry.	Mixed with diet formulation	[16]
Weaned piglets	*Lactobacillus reuteri*, *Bacillus subtilis* and *Bacillus licheniformis*	Digestibility of dry matter, crude protein, and crude fat increased upon treatment with probiotic and significant reduction of fecal Salmonella and *E. coli* counts with an increase of probiotics counts.	Mixed with diet formulation	[17]
Broiler chicks	*Lactobacillus acidophilus* (LASW), *L. fermentum* (LF33), *L. plantarum* (LPL05), and *Enterococcus faecium* (TM39)	LAB administration showed effectiveness in antagonistic effect against *Salmonella* colonization, invasion, and the induced inflammation.	Mixed with diet formulation	[18]
Dairy cows	Propionibacterium, *S. cerevisiae*, *L. acidophilus*, *L. ecidophilus*, *L. casei* and *Enterococcus faecium*	Significantly improves milk yield as well as the milk composition.	Direct-feds microbials	[19]
Crossbred cows	*L. acidophilus*, *S. cerevisiae*, *S. boulardii* and *Propionibacterium frendenreichii*	Probiotics proved to be effective in increasing milk production of lactating cows. Milk fat, milk protein and solid-not-fat content tended to be higher in cows supplemented with probiotics.	Direct-feds microbials	[20]
Agriculture				
Wheat	*Enterobacter*, *Serratia*, *Microbacterium*, *Pseudomonas* and *Achromobacter*	Halotolerant bacterial consortia significantly ($P \geq 0.05$) increased the emergence, growth, biomass and Super Oxide Dismutase activity of wheat seedlings exposed to salt stress.	Biofertilizers	[21]

Subject	Probiotics organism	Results	Application	Reference
Cotton	Rhizobacteria	The bacterial inoculum (50 g/kg of seed) significantly increased seed cotton yield (21%), plant height (5%) and microbial population in soil (41%) over their respective controls	Biofertilizers	[22]
Arabidopsis and cucumber (*Cucumis sativus* L.)	*Trichoderma asperelloides* T203	*Trichoderma* spp. stimulate plant growth prior to salt stress imposition and significantly improved seed germination.	Biofertilizers	[23]
Iceberg lettuce (*Lactuca sativa* L.) and rocket (*Eruca sativa* Mill.)	Trichoderma strains: *T. virens* (GV41) and *T. harzianum* (T22)	Trichoderma able to manage the nutrient content of leafy horticulture crops cultivated in low fertility soils, and assist vegetable growers in reducing the use of synthetic fertilizers, and optimize N use efficiency.	Biofertilizers	[24]
Saffron (*Crocus sativus* L.)	*R. intraradices* and *Funneliformis mosseae*	Soilless cultivation systems enhanced by the symbiosis with arbuscular mycorrhizal fungi able to produce high quality saffron.	Biofertilizers	[25]

Table 1.
Reports of the use of probiotics in aquaculture, animal husbandry, and agriculture.

of probiotic microorganisms that are able to dissolve the P element in acid soils can increase the production of agricultural commodities as reported by many researchers [21–25].

3. Bioremediation: healthy environment healthy life

Application of microorganisms, in this case, bacteria to improve the environment is actually not only probiotics but also the bioremediation process. In the bioremediation, microorganisms are also used to neutralize, detoxify, remove, clean up, break down, and/or decompose waste and other hazardous pollutants into less toxic or non-toxic substances. Microorganisms in the aquaculture environment are in direct contact with animals, with gills and food supplied, having easy access to the digestive tract of animals. Among the microorganisms that exist in the aquatic environment are microorganisms that are potentially pathogenic, opportunistic,

Figure 1.
Aerial image of Cirata dam located in West Java, Indonesia (upper) showing uncontrolled density of floating cage aquaculture causing rapid degradation in its water quality (lower-left); deaths of thousands of tons of fish in floating cages aquaculture due to lack of oxygen and rising toxic gas (lower-right) causing economic loss of approx. USD 28.5 million [29]. Image courtesy of Google maps [31–33].

those who take advantage of situations of animal stress in high stocking density, poor nutrition to cause infections, low growth and feed efficiency rates, and even death (**Figure 1**).

Mass fish deaths often occur in Indonesian waters with the greatest frequency and quantity occurring in lake/reservoir waters, followed by rivers and finally in sea/coast waters. The most common cause of mass death of fish is hypoxia, mainly due to the up-welling phenomenon, namely the increase in the mass of the bottom water to the surface, so that the waste generated from aquaculture activities in the form of organic material rises to the surface [26]. Up-welling events usually take place in the rainy season which results in differences in water density between surface water and bottom waters. Rising water will mix and carry organic materials and toxic gases (such as H_2S and ammonia) that can cause poisoning to fish or cause a decrease in dissolved oxygen levels due to plankton blooming that is triggered by excess of nitrate and phosphate nutrients. In marine waters, mass fish deaths have also occurred, such as in November 2015 in Jakarta Bay or earlier in 2004 and 2005, and in Lampung Bay in 2013. The mass deaths of sea fish are usually caused by the

phenomenon of algal blooming (red tide), namely the plankton population explosion that was followed by mass death and caused a decrease in oxygen content due to algal respiration processes [27, 28]. As a result, fish become oxygen deficient or there is a blockage in the fish's respiratory organs (gills) by plankton. Besides causing economic losses because most of the fish that die suddenly cannot be utilized anymore, the remaining fish carcasses also pollute the environment due to their suboptimal handling. Potential economic loss of aquaculture sudden death in Cirata dam incident in 2013 is IDR 427,6 Billion or USD 28.5 Million [29]. For this reason, the use of probiotics for aquaculture aims not only at direct benefits for animals but also their effects on the fishing environment. There are two types of probiotic applications, first with direct feed (mixed into diet formulation) and secondly through the environment (mixed with water and/or sediment). Application of the first method can improve feed quality by adding additives in the form of probiotics containing beneficial microbes and decomposers into the feed that can function to improve feed quality by converting them into compounds that are more easily absorbed by the intestine thereby increasing feed digestibility. While the second application will improve the quality of the surrounding environment, e.g., decompose toxic substance such as ammonia, sulphide, fish excreta, also excess feed that potentially attract pathogens and other unwanted micro- and macroflora. Benefits observed in probiotic supplementation in aquaculture include:

1. Increasing the nutritional value of food and its absorption by increasing the extent of the absorption area;

2. Increased activity of digestive enzymes;

3. Factors driving growth;

4. Pathogens inhibition;

5. Increased immune response; and

6. Improving the quality of fisheries water.

Meanwhile, one way to improve soil environmental quality is by introducing microbes to the soil through compost. Making compost from organic materials, in the form of by-products of agricultural activities as well as household, market and municipal waste needs to be encouraged. More effective compost making technology can be done by using a starter or microbes that accelerates the decomposition of organic material which is beneficial for plants. Giving compost on agricultural land provides a double benefit, namely improving soil microbial composition and increasing soil organic matter content. In order to make effective use of microbes in improving agriculture, animal husbandry and fisheries, it is necessary to develop microbial cocktails for each species of plant, livestock or fish [30]. The cocktail must have key features such as:

1. Able to be prepared on a large enough scale,

2. Remain stable and viable for a long time,

3. Good viability and growth, and

4. Give a positive impact on the host.

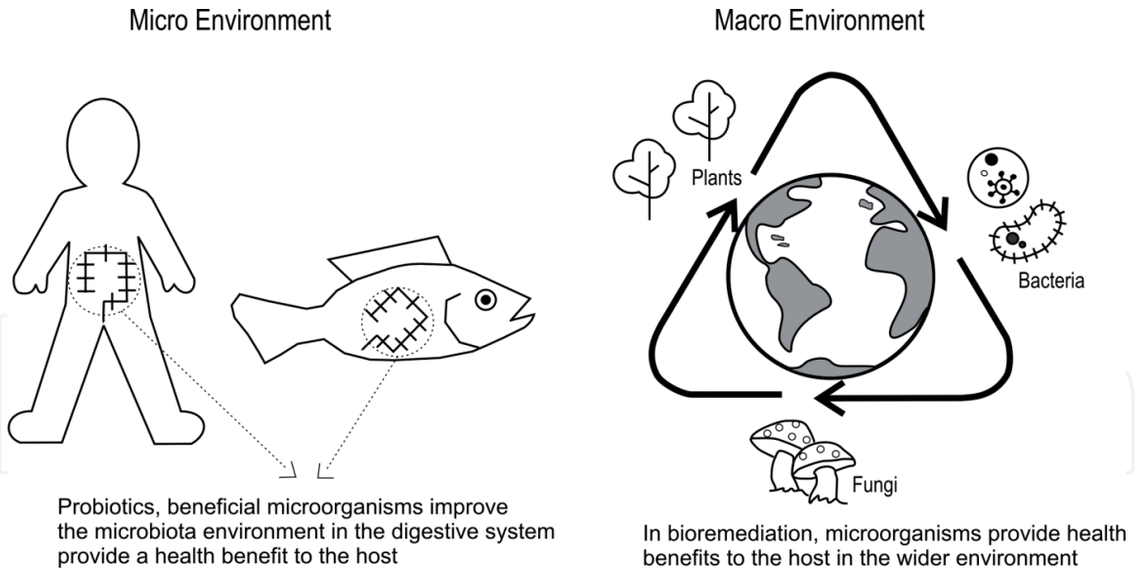

Figure 2.
The relationship between probiotics and bioremediation on a broader scale.

The working mechanism of bioremediation involves several technical aspects such as biotransformation, biodegradation, mineralization, phytohydraulics, bioaccumulation, and biovolatilization where the degrading microbes or plants remove, transform, modify, and/or convert a complex compound of pollutants into simpler and less toxic compounds. This bioremediation system has been successfully applied in cleaning contaminated sites, agricultural land, sediments, ground water, surface water, and sea water. Bioremediation through microorganisms generally involves the application of aerobic and anaerobic bacteria and fungi to restore the environment. Rhizoremediation is a remediation technique of soil contaminated pollutants by the action of plants (phytoremediation) and their symbiotic rhizosphere microbes. Plant growth-promoting microbes have been used for the restoration of infertile marginal land by increasing crop productivity. The application of probiotics on a broader scale is bioremediation. **Figure 2** shows the relationship between probiotics and bioremediation, where probiotics are defined as living microorganisms which, if given in sufficient quantities, provide health benefits to the host (humans, and/or animals), while bioremediation is augmentation or stimulation of microorganisms in sufficient quantities and manners, providing health benefits to the host (remediate or restoring polluted environment).

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